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Cates,
R. G.

Submitted to Nature, 7 Dec 1982

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Stress physiology, patterns in defensive chemistry,
and spruce budworm success

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Abstract

Hypotheses concerning budworm success, and changes in foliage quality due to water stress, were tested using experimentally stressed Douglas-fir trees. On the stressed trees, more larvae survived, and the resulting females were heavier. Budworm success was most strongly associated with changes in the defensive terpene chemistry of the foliage than with the primary nutrition. These data suggest that increases in insect populations could occur quickly given changes in host plant quality due to abnormally stressful conditions.

A considerable body of literature is available concerning factors that may increase the susceptibility of plants to herbivores. Most often cited is the relationship between climate or weather and population fluctuations of phytophagous insects¹⁻⁸. Specifically, the idea of a 'climatic release' of an endemic population following a severe stress has been advanced.

Stresses, such as drought, prolonged high temperatures, water-logged and nutrient deficient soils, diseases, old age, and level of maturity have been implicated as contributory to insect outbreaks. Outbreaks, however, do not always occur on such sites and stands⁴⁻⁷. This discrepancy, and the fact that numerous biotic and abiotic factors predispose insects to outbreaks, have brought into question aspects of the climatic release hypothesis and have led to a greater focus on the changes that may be occurring in host and foliage quality due to abnormally stressful factors. For example, commonly shown is a significant relationship between changes in nitrogen within plant tissues and the growth, survival, and reproductive success of phytophagous insects⁹. Plants growing under abnormally stressful conditions are known to exhibit complex changes in nitrogen form and content¹⁰⁻¹³. These considerations led White^{6,14} to suggest that a major cause of outbreaks of psyllids and other phytophagous insects is the change in nitrogen levels and composition in the tissues of water stressed host plants.

Rhoades⁴ postulates that, in addition to nutritional changes, there are significant changes in the natural product defensive systems of stressed plants. The suggestion is that, in general, toxin and digestibility-reducing defenses are altered quantitatively, and possibly qualitatively, resulting in an imbalance between primary nutrients and

secondary metabolites that are postulated to favor insect growth, survival, and reproduction.

Over the past several years, we have investigated the effects of drought stress on host tree foliage quality and western spruce budworm (Choristoneura occidentalis - Tortricidae) success. Our objectives were to determine if budworm feeding on Douglas-fir (Pseudotsuga menziesii-Pinaceae) growing under different levels of water stress enjoyed greater success (as measured by survival and adult dry weight) on stressed trees, and whether there were any differences in foliage chemistry and tree physical parameters that were associated with observed insect success. Our hypotheses concerning tree stress and budworm success, were tested using trees growing on north and south facing slopes within the Santa Fe National Forest near Jemez Springs, New Mexico, USA.

Two sites were selected such that differences in water availability would be maximized. Experimental trees on both sites were selected such that the variability in age, height, dbh, foliage chemistry, and microenvironmental conditions other than water availability were minimized. Trees growing on the north facing slope (non-stress; n = 11) were hypothesized to be under less water stress than trees growing on the south facing slope (stressed; n = 17). Additionally, at the stress site, roots growing in approximately a third of the area beneath each tree were trenched to a depth of 0.5 m (approximately 1 m from the tree bole) in an effort to maximize water stress. A significant root area under each tree was not trenched so that minimal physiological damage was done to each tree. After the trees were trenched, budworm larvae were collected from the nearest infested site and sorted to third instar. Five of these larvae were placed in each of 5 screen bags in the midcrown on the north side of each tree at

both sites. After pupation, the budworm were counted, brought to the laboratory, and placed in a controlled environmental chamber. As the adults emerged they were sexed, dried, and weighed. Young needles (current year's growth) were collected twice; once prior to trenching and a second time while the larvae were in the 4th and 5th instar. Because of the large amount of variability in terpene chemistry among the 3 crown levels of the trees, tissue was randomly collected from the same midcrown area where the larvae were reared. Thirty mg of this tissue were analyzed for terpenes using a Perkin-Elmer capillary column (85% OS-138, 14% CO-880, 1% V-930). Terpene methods are outlined in detail in Redak¹⁵. Methods for protein complexing capacity were modified after Feeny¹⁶ and Feeny and Bostock¹⁷. Nitrogen was determined by standard microkjeldahl methods. Xylem water potentials were determined using a portable Scholander pressure bomb. Complete details of any of these methods can be obtained from the authors. The foliage of trees from both sites was analyzed for 13 individual terpenes, protein complexing capacity, and total and soluble nitrogen. These variables plus the total terpene content and tree age were used in a stepwise discriminant analysis to determine if foliage chemistry differed between the 2 sites (a total of 18 variables).

Differences between sites in quantities of terpenes, total and soluble nitrogen, and protein complexing capacity prior to trenching were analyzed using Wilcoxon signed rank test. The results showed that the concentrations were significantly higher in the foliage from trees on the stressed site, which biased against finding any increased insect success related to chemical changes. A further bias against larger budworm being found on the stressed site, is the suggestion by Precht et al.¹⁸ that larger final size is associated with lower temperatures. Temperatures at

the non-stressed site were up to 2°C cooler than the stressed site during the experiment.

To successfully test our hypotheses we needed to first demonstrate that the degree of water stress was different among trees between the two sites. Xylem water potentials, measured after trenching but during the time that the budworm were on the trees, averaged 23% higher for the stressed trees as compared to the non-stressed trees (t-test; $p < 0.001$).

Next, we determined if the number of surviving larvae, and the male and female adult dry weights, were different between the two sites. Preliminary analysis showed no dependence between adult dry weight and the number of survivors per tree ($r = 0.158$; $p = 0.12$). Consequently, these variables were analyzed using multivariate analysis of variance to test for the effects of site and sex. The cell means for each of the variables are given in Table 1a. Table 1b gives the results of the multivariate analysis. The relative contributions of numbers of survivors and weight to the differences between sites and sexes are indicated by the characteristic vector coefficients. As expected, differences between sexes were due exclusively to differences in weight between the dimorphic males and females. More interesting were the differences between sites in both the number of surviving larvae and adult dry weights. The numbers of female larvae surviving per tree (5.2 vs 3.0) and their dry weights (23.6 vs 18.2 mg) were significantly higher on the stress site compared to the non-stress site. No interaction between site and sex was detected.

The final step was to determine, using stepwise discriminant analysis, which, if any, of the tree or foliage parameters differed between sites. This analysis indicated that the concentrations of only 7 of the initial 18 variables differed significantly, and that these completely differentiated

the sites ($F_{(7,193)} = 210.4$; $p < 0.0001$). The remaining 11 variables failed to enter into the discriminant function. Examination of the standardized discriminant function coefficients of the included variables suggested that the differences between sites were due almost exclusively to monoterpene chemistry (Table 2). The discriminant function contrasted the relative concentration of α -pinene in stressed trees versus the concentration of several terpenes, particularly bornyl acetate and β -pinene in non-stressed trees. A histogram of the discriminant scores for the trees (Figure 1) shows that the stress site trees load negatively on the function while the non-stressed trees load positively. This indicates that stressed trees contain higher levels of α -pinene while the non-stressed trees contain higher levels of bornyl acetate, β -pinene, and other terpenes in the foliage.

Our data suggest that budworm reared on stressed trees not only were larger, but more of them survived. Elsewhere, we have shown that western spruce budworm female dry weight is correlated significantly with the number of eggs produced per female¹⁵. Consequently, we concluded that heavier females on the stress site were more fecund. The increased survival and fecundity suggest that on stressed trees the result may be a major increase in budworm populations in succeeding generations. Therefore, we expect that increases in budworm, as well as other phytophagous insect populations, could occur in a short time given abnormally stressful conditions for the host plants.

While it seems clear that nitrogen, water, and other primary nutrients are essential to successful growth and reproduction, changes in defensive natural product chemistry also has an affect on insect success^{1,19,20}. The results presented here are consistent with this in that the quantities

of bornyl acetate, β -pinene, and other terpenes were reduced in the foliage of trees on the stress site to levels lower than those of the trees on the non-stress site--levels significantly lower than the pretreatment condition of the foliage. This change in foliage quality was associated with a 73% increase in larval survival, and a 30% increase in female biomass. It is difficult to determine the direct effects of the interaction of soluble nitrogen and terpenes on western spruce budworm success. The coefficient for soluble nitrogen is small in comparison to those for the terpenes suggesting that soluble nitrogen is not as important as are the terpenes in determining budworm success. While small changes in soluble nitrogen may have a significant influence on budworm success, these data suggest that stress-induced changes in defensive chemistry are major factors influencing budworm survival, biomass production, and fecundity.

We thank J. Gray Hubler, S. Andrews, D. Potter, H. Alexander, and W. McConnell for their invaluable help. Work leading to this publication was funded in part by the Canada/United States Spruce Budworms Program, and Accelerated Research, Development, and Applications Program, sponsored by the USDA Forest Service. This work, also, was partially supported by NSF grant DEB 76-19950.

Figure 1. Histogram of discriminant scores for stressed and non-stressed trees. Negative scores indicate higher levels of α -pinene and soluble nitrogen. Positive scores indicate higher levels of bornyl acetate, β -pinene, unidentified terpenes 1 and 2, and tree age.

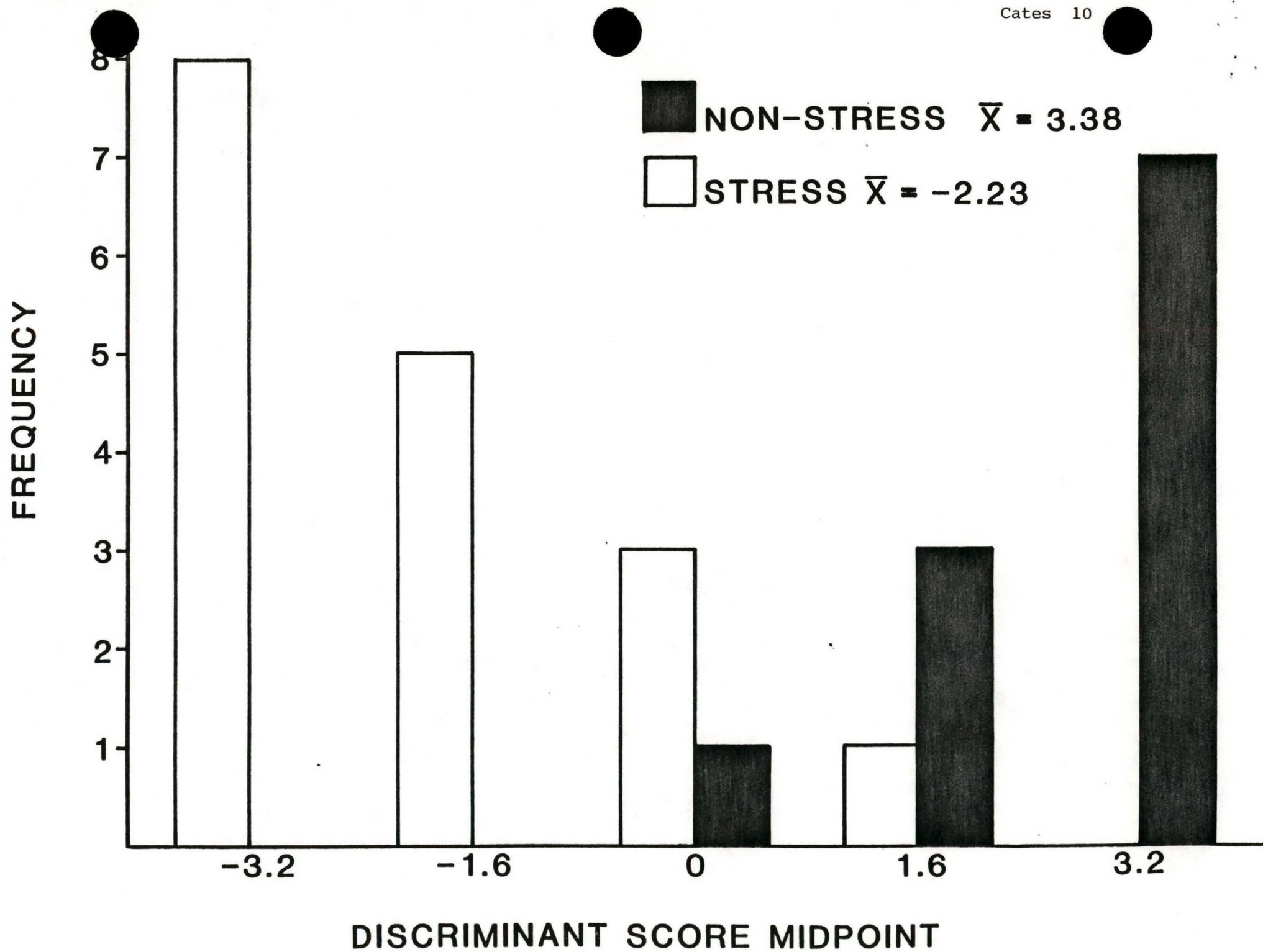


Table 1. Multivariate analysis of variance for site and sex effects on adult weight and number of surviving budworm. a) Mean adult dry weight and number of budworm surviving per tree on stressed and non-stressed sites; b) MANOVA results; characteristic vector coefficients indicate the relative contribution of the dependent variables to a particular effect.

a)

Site	Sex	N	Weight (mg)	Number Survived
Non-stressed	Male	22	9.0	3.7
	Female	23	18.2	3.0
Stressed	Male	27	10.7	4.6
	Female	26	23.6	5.2

b)

Source	Wilks'	F(2,93)	Characteristic Vector	
	Lambda		Coefficients	
			Number	Weight
Site	0.81	10.78*	0.022	0.018
Sex	0.38	73.97*	-0.007	0.023
Site x Sex	0.95	2.42 ^{NS}	0.018	0.019

* $P \leq 0.0001$; NS = not significant at 0.05 level

Table 2. Coefficients for the 7 variables resulting from the analysis of chemical and physical parameters among trees growing on the stressed and non-stressed sites.

Variable	Standardized Discriminant
	Function Coefficient
α -pinene	-5.84
Soluble nitrogen	-0.71
Age	0.34
Unidentified terpene 1	1.22
Unidentified terpene 2	1.66
β -pinene	2.57
Bornyl acetate	2.81

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